NOTE TO THE FILE
BNF0056
September 28, 1998

Subject: Glyphosate-Tolerant Sugar Beet Line 77

Keywords: Sugar beet (*Beta vulgaris*), 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), *Agrobacterium* sp. strain CP4, glyphosate oxidoreductase (GOX), *Ochrobactrum anthropi*, glyphosate tolerant, herbicide tolerant, β-D-glucuronidase (GUS)

Background

On June 5, 1998, Monsanto Company and Novartis Seeds, Inc. discussed with FDA their safety data for the GOX-sugar beet fusion protein (Protein 34550) that is present in their new glyphosate-tolerant sugar beet. Following the discussion, Monsanto and Novartis provided FDA with a summary of the safety and nutritional assessment they have conducted on the new glyphosate-tolerant sugar beet line 77.

Intended Effect and Food/Feed Use

The intended effect of the genetic modification is to make sugar beet (*Beta vulgaris*) plants tolerant to the non-selective herbicide glyphosate. Glyphosate's herbicidal activity is conferred by its ability to potently inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). The EPSPS enzyme catalyzes the conversion of shikimate-3-phosphate (S-3-P) and phosphoenolpyruvate (PEP) into 5-enolpyruvylshikimate-3-phosphate (EPSP), an intermediate in the production of aromatic amino acids that takes place in the chloroplast. The EPSPS enzyme from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) is highly resistant to inhibition by glyphosate. Monsanto and Novartis used the *Agrobacterium* sp. strain CP4 EPSPS gene to confer tolerance to the herbicide in their transgenic sugar beet.

Sugar beets are grown for the fleshy, bulbous root, which normally contains 10-15% sucrose. Sugar beets are processed into products for use in both human and animal food. Sugar, which is used in a variety of foods, is the main product. Common by-products from sugar processing are sugar beet molasses, which is used for production of yeast, chemicals, pharmaceuticals, and mixed cattle feeds; dried sugar beet pulp, which is used in feeding dairy cattle, beef cattle, and sheep; and sugar beet fiber, which is used in human food as a source of fiber. A very modest amount of sugar beet tops is consumed as animal feed.

Molecular Alterations and Characterization

Monsanto and Novartis transformed sugar beet line A1012 with a disarmed *Agrobacterium tumefaciens* double border plant transformation plasmid to produce their glyphosate tolerant sugar beet line 77. The plasmid contained the following genes within the T-DNA border sequences of the vector: (1) the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) gene from *Agrobacterium* sp. strain CP4; (2) the *uidA* gene, which encodes β-D-glucuronidase (GUS), from *E. coli*; (3) a glyphosate oxidoreductase gene (*gox*) from *Ochrobactrum anthropi*; and (4) a neomycin phosphotransferase gene (*nptII*) from transposon *Tn5* which provides resistance to aminoglycoside antibiotics, such as neomycin and kanamycin. In addition, the plasmid contained a bacterial selectable marker gene, *aad*, which provides resistance to spectinomycin and streptomycin, as well as two origins of replication (*ori-V* and *ori-322*) to permit replication and maintenance of the plasmid in bacteria.

Typically only those DNA sequences within the left and right border sequence are transferred and integrated into the plant chromosome. The firms performed molecular analyses to characterize the integrated DNA. According to the firms, Southern blot analyses revealed that one intact copy of both *cp4 epsps* and *gus* (or *uidA*) transferred to the sugar beet genome, along with a truncated copy of *gox*. By sequencing, the firms identified two potential translation stop codons in the sugar beet DNA 130 base pairs (bp) and 234 bp downstream from the junction between the T-DNA and sugar beet DNA. At position 897 bp downstream of the *gox* start codon, the *gox* gene was truncated. Approximately 69% of the N-terminal portion of *gox* is present in the sugar beet genome. The firms report that sequencing data and Southern blot analyses show *nptII* to be absent from the genome of line 77. Because *aad* as well as *ori-V* and *ori-322* were outside the T-DNA region of *Agrobacterium* plasmid, the firms did not anticipate the integration of *aad* in the genome of line 77. By Southern blot analyses, the firms confirmed that these DNA sequences were absent in the genome of line 77.

Segregation studies performed by the firms indicate that glyphosate tolerance and the GUS expression are stably inherited dominant traits. Each trait appears to be at a single locus. To ascertain whether the foreign DNA in line 77 was stably integrated into the genome, the firms performed Southern blot analyses on DNA from generational descendants (F2, F3 and F4) of the original transformant. The firms state that these analyses demonstrate that the inserted DNA, containing the full length *cp4 epsps* and *uidA*, and the truncated *gox*, has not been altered during the breeding process.

Expression of CP4 EPSPS and GOX are controlled by the figwort mosaic virus (FMV) promoter and targeted to the chloroplast by a chloroplast targeting sequence from *Arabidopsis thaliana*. Expression of GUS is controlled by the cauliflower mosaic virus 35S promoter.

Expressed Proteins

The glyphosate-tolerant sugar beet line 77 contains three introduced proteins: CP4 EPSPS, the selectable marker GUS and the GOX-sugar beet fusion protein, which the firms refer to as Protein 34550. The firms determined that Protein 34550 is composed of 89 amino acids from the chloroplast transit peptide (CTP1), 299 amino acids of the N-terminus of GOX and 43 amino acids encoded by sugar beet genomic DNA. Using ELISA or western blot analyses, the firms determined the amount of Protein 34550 expressed in line 77. The level of Protein 34550 in tops (leaf tissue) was 0.004µg/mg fresh weight or 4 ppm, and the level in root tissue was lower than in the tops. The level of CP4 EPSPS and GUS were measured in beet samples collected from the 1995 harvest in Europe (6 locations) and the 1996 harvests in the U.S. (5 locations) and Europe (6 locations). The corresponding mean levels of CP4 EPSPS were 0.285, 0.172 and 0.190 µg/mg fresh weight in tops, and 0.054, 0.047 and 0.063 µg/mg fresh weight ~~from~~ in brei, a form of processed sugar beet root. The mean levels of GUS were 0.0030, 0.00278 and 0.0034 µg/mg fresh weight in tops, and 0.0006, 0.00039 and 0.0005 µg/mg in brei. The levels of CP4 EPSPS and GUS were also measured in early leaves from the 1995 harvest. The mean values were 0.145 µg/mg and 0.002 µg/mg for CP4 EPSPS and GUS, respectively. All levels are expressed on the basis of fresh tissue weight.

Safety of the Expressed Proteins

The firms state that the major traits of allergens are sequence homology to known allergens, resistance to gastrointestinal digestion and high concentrations of the protein in foods that elicit an allergenic response. According to the firms, the expressed three proteins, CP4 EPSPS, GUS and Protein 34550, were not obtained from sources known to be allergenic. Using public domain genetic databases (including GenBank, PIR, and SwissProt), the firms report that no significant homology was found for CP4 EPSPS, GUS, and Protein 34550 to any of the known allergens in these databases. All three proteins were extremely labile to digestion by proteases present in the mammalian digestive system. All three proteins are expressed at low levels; those for CP4 EPSPS and GUS are approximately 0.005% and 0.00006% of root fresh weight. For Protein 34550, the amount in leaf tissue is 0.004 µg/mg fresh weight.

The firms report that the three expressed proteins also lack homology with known toxic proteins, and lack acute toxicity to mammals. The firms also report that the proteins are expressed at low levels in the sugar beet itself and the amounts of proteins are not detectable or at low levels in processed sugar beet food and feed products. From this information, the firms determined that dietary exposure to these proteins would be extremely low. The firms conclude that the proteins introduced into their transgenic sugar beet are not potentially harmful to humans and animals.

Compositional Analyses

The firms performed compositional analyses on sugar beets obtained from a number of field trials conducted in both the U.S. and Europe. The firms conducted proximate analyses on top and root samples for ash, crude fiber, crude protein, dry matter, soluble carbohydrates. In addition, crude fat was measured in tops, and acid detergent fiber and neutral detergent fiber were measured in roots. The firms report that all values are comparable between the control and transgenic sugar beet and fall within literature ranges. The firms also analyzed processed sugar beet (brei) for sucrose, sodium, potassium, amino nitrogen and invert sugar levels. The firms report that all values are comparable between the control and the transgenic sugar beet and within the literature value ranges.

Saponins are triterpenoid glycosides that have a broad biological activity and occur naturally in numerous food and feed crops including sugar beets. The firms conducted analyses for saponins in root and top tissues from the transgenic and control sugar beets. From their analyses, the firms conclude that the levels of saponins in their transgenic sugar beet are not altered relative to commercially available varieties, and the levels of saponins in the transgenic sugar beet fall within the reported literature range for traditional sugar beet lines.

Conclusion

Monsanto and Novartis have concluded that their transgenic sugar beet line 77 is not materially different in terms of food safety and nutritional profile from sugar beet varieties currently on the market. At this time, based on Monsanto's and Novartis' description of their data and analyses, the Agency considers the consultation on their glyphosate-tolerant sugar beet line 77 to be complete.

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